the actin-myosin interaction using in vitro motility and laser beam trapping assays. From the in vitro motility assay, we measured the maximum velocity from wt α- and β-S1. Using the laser trap, we measured stroke sizes, ATP binding rates (low [ATP]) and ADP release rates (high [ATP]). Furthermore, we expressed several HCM (R403Q, S453C) and DCM (S532P) causing mutants, and obtained preliminary in vitro motility and trap data. We have built a modern version optical trap that can resolve the ~10 nm stroke size and ~10 ms strongly bound state of cardiac β-S1 at high [ATP]. We have further improved the resolution by implementing real-time feedback control in the system to accurately determine fine changes caused by the single mutations.

3118-Plat
Cross-Bridge Kinetics in Papillary Muscle Fibers from Transgenic Mice Expressing the Aspargine-47 to Lysine (N47K) Mutation in Myosin Regulatory Light Chain
Li Wang[1], Priya Muthu[2], Danuta Szczesna-Cordary[2], Masataka Kawai[1].
[1]University of Iowa, Iowa city, IA, USA, [2]University of Miami, Miami, FL, USA.
The current study aimed at investigating the isometric tension, stiffness and elementary steps of the cross-bridge (XBr) cycle in skinned papillary muscle fibers from transgenic mice expressing familial hypertrophic cardiomyopathy (FHC) associated mutation N47K. This mutation, located at the Ca^{2+} binding site of myosin regulatory light chain (RLC), is associated with a rapidly progressing late onset of the disease with mid-ventricular and papillary muscle hypertrophy. We studied the effect of the N47K mutation on the stiffness and the kinetic constants using skinned muscle strips subjected to increasing concentrations of MgATP, phosphate (Pi), and Ca^{2+} at 200 mM ionic strength, pH 7.0, and 20°C. The kinetic constants of the XBr cycle and their distribution over 6 states were deduced from the sinusoidal analysis. We found that active tension was higher in N47K fibers compared with WT. No mutation-mediated significant changes were observed in the Ca^{2+} sensitivity (pCa_{50}) or cooperativity (n_H). The apparent rate constants $2\pi b$ and $2\pi c$ were smaller in N47K fibers compared with WT. The rate constant of XBr detachment step (k_2) was smaller while the equilibrium constant of force generation (K_4) was larger in N47K fibers. The stiffness of the standard activation and the number of the strongly attached XBr were larger in N47K than in WT.These results suggest that the higher tension in N47K fibers may be related to a larger number of strongly attached XBr. We conclude that the N47K mutation may initiate a hypertrophic response through changes in myosin XBr kinetics leading to increased force during the systole, which eventually results in a pathologic phenotype in patients carrying this FHC-linked RLC mutation. Supported by NIH-HL070041 (MK), NIH-HL071778 and HL090786 (DSC) and AHA 10POST3420009 (PM).

3119-Plat
Myosin Cross-Bridges do not Form Precise Rigor Bonds in Hypertrophic Heart Muscle Carrying Troponin T Mutations
Krishna Midde[1], V. Dumka[1], JR Pinto[2], P. Muthu[2], P. Marandos[3], I. Gryczynski[1], Z. Gryczynski[3], J. Borejdo[1].
[1]University of North Texas Health Science Center, Fort Worth, TX, USA, [2]University of Miami, Miller School of Medicine, Miami, FL, USA, [3]Texas Christian University, Fort Worth, TX, USA.
Distribution of orientations of myosin was examined in *ex-vivo* myofibrils from hearts of transgenic (Tg) mice expressing Familial Hypertrophic Cardiomyopathy (FHC) troponin T (TnT) mutations I79N, F110I and R278C. Humans are heterozygous for sarcomeric FHC mutations and so hypertrophic myocardium contains a mixture of the wild-type (WT) and mutated (MUT) TnT. If mutations are expressed at a low level there may not be a significant change in the global properties of heart muscle. In contrast, measurements from a few molecules avoid averaging inherent in the global measurements. It is thus important to examine the properties of only a few molecules of muscle. To this end, the lever arm of one out of every 60,000 myosin molecules was labeled with a fluorescent dye and a small volume within the A-band (~ 1 fL) was observed by confocal microscopy. This volume contained on average 5 fluorescent myosin molecules. The lever arm assumes different orientations reflecting different stages of actomyosin enzymatic cycle. We measured the distribution of these orientations by recording polarization of fluorescent light emitted by myosin-bound fluorophore during rigor and contraction. The distribution of orientations of rigor WT and MUT myofibrils was significantly different. There was a large difference in the width and of skewness and kurtosis of rigor distributions. These findings suggest that the hypertrophic phenotype associated with the TnT mutations can be characterized by a significant increase in disorder of rigor cross-bridges.

3120-Plat
Mutations in thin Filament Proteins that Cause Familial Dilated Cardiomyopathy Uncouple Troponin I Phosphorylation from Changes in Myofibrillar Ca^{2+}-Sensitivity
Massimiliano Memo, Andrew E. Messer, Man Ching Leung, Steven B. Marston.
Imperial College London, London, United Kingdom.
To investigate the molecular mechanism leading to familial dilated cardiomyopathy we studied 7 different DCM-causing mutations in thin filament proteins by *in vitro* motility assay. Troponin I K36Q and troponin C G159D mutant troponin was extracted from explanted human hearts. Troponin T ΔK210 and R141W mutant troponin, and actin E361G were extracted from transgenic mouse heart. Mutant α-tropomyosin E40K and E54K was expressed in baculovirus/*sf9* cells. We reconstituted thin filaments *in vitro* with the mutant sample of interest, or its correspondent wild-type, combined with troponin or α-tropomyosin from human donor tissue. We could not observe any common pattern for the absolute Ca^{2+}-sensitivity of the mutants compared with the WT thin filaments. In contrast to this we consistently observed that DCM mutations affect the relationship between Ca^{2+}-sensitivity and troponin I phosphorylation by PKA. Normally, PKA phosphorylation of troponin I causes a 2-3 fold decrease in myofibrillar Ca^{2+}-sensitivity but we found that, in thin filaments containing DCM mutations, Ca^{2+}-sensitivity did not change with the level of troponin I phosphorylation. Uncoupling of Ca^{2+}-sensitivity from troponin I phosphorylation was observed for all DCM mutations tested irrespective of the gene mutated or the source of the mutant protein. We tested the DCM mutation dose-dependency by mixing α-tropomyosin E40K, with the wild-type in different ratios, assembling it into thin filaments and comparing Ca^{2+}-sensitivity when troponin I was phosphorylated and dephosphorylated. The result was a dramatic effect of uncoupling with small ratios of the mutant protein with complete uncoupling at 50% mutant. We conclude that the causative property shared by mutations in contractile proteins that cause DCM is a blunted response to changes in troponin I phosphorylation that could impair the normal response to adrenergic stimulation.

3121-Plat
Post-Translational Modifications of Myofilament Proteins Involved in Length-Dependent Prolongation of Relaxation in Rabbit Right Ventricular Myocardium
Michelle M. Monasky[1], Domenico M. Taglieri[2], Alice K. Jacobson[1], Kaylan M. Haizlip[1], R John Solaro[2], **Paul Janssen**[1].
[1]The Ohio State University, Columbus, OH, USA, [2]University of Illinois at Chicago, Chicago, IL, USA.
The phosphorylation state of several cardiac myofilament proteins changes with the level of stretch in twitch-contracting cardiac muscles. It remains unclear which kinases are involved in the length-dependent phosphorylation of these proteins. Therefore, we set out to investigate which kinases are involved after a step-wise change in cardiac muscle length. We hypothesize that myofilament protein phosphorylation by PKCβII and PKA alter contractile kinetics during length-dependent activation. Right ventricular intact trabeculae were isolated from New Zealand White rabbit hearts and stimulated to contract at 1 Hz. Twitch force recordings where taken before and after administration of kinase inhibitors at varying muscle lengths at 37°C. PKCβII inhibition significantly decreased time from stimulation to peak force (TTP), time from peak force to 50% relaxation (RT50), and time from peak force to 90% relaxation (RT90) at optimal muscle length. However, developed tension did not significantly change at either muscle length studied. Non-specific PKA inhibition significantly decreased TTP and RT50 at both taut and optimal muscle lengths. Detection of Ser/Thr phosphorylation with ProQ-diamond staining indicates a role for PKCβII in the phosphorylation of tropomyosin and myosin light chain-2 (MLC2) and PKA for tropomyosin, troponin-I, and MLC2. Our data provide evidence for two signaling kinases which act upon myofilament proteins during length-dependent activation, and provide further insight for length-dependent myofilament function.

3122-Plat
Transmural Distribution of Myocyte Contractile Properties in Aging F344 Rats
William K. Snapp[1,2], Stuart G. Campbell[1,2], Kenneth S. Campbell[1,2].
[1]Department of Physiology, University of Kentucky, Lexington, KY, USA, [2]Center for Muscle Biology, University of Kentucky, Lexington, KY, USA.
Age-associated diastolic heart failure occurs when the amount of blood filling the heart between beats is reduced due to slow relaxation and increased passive stiffness of the left ventricle. We are testing the hypothesis that slowed active relaxation in diastolic heart failure is caused in part by age-dependent